Conditional Removal of Myeloid Cell-Derived *Mif* through a Cre-loxP System in Mice

Research Thesis

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by

Arham Siddiqui

The Ohio State University

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Project Advisor: Dr. Steve Oghumu, PhD, Department of Pathology

Abstract
Oral cancer affects people globally with an estimated 657,000 new cases and 330,000 deaths yearly. *Macrophage migration inhibitory factor* (*Mif*) is a gene that encodes for a cytokine involved in regulating cell-mediated immunity, immunoregulation, and inflammation. Previous research has demonstrated that global *Mif* inhibition results in reduced oral cancer development. This was associated with a decrease in the accumulation of myeloid cell populations in the oral tumor microenvironment. It is therefore important to elucidate the role of *Mif* expression by myeloid populations in the oral tumor microenvironment. We previously generated floxed *Mif* reporter mice (*Mif* $^{Fl/Fl}$). Selective breeding between *Mif* $^{Fl/Fl}$ mice and *Cre* knock-in mice (which contains a nuclear localized cre recombinase targeted to the *Lysozyme* 2 gene locus) (*LysMCre*) was performed to produce mice with *Mif* deficiency in myeloid cells. Genotypic characterization for Mif $^{Fl/Fl}$ and *LysMCre* was performed by PCR of genomic DNA extracted from mouse ear tissue. Phenotypic characterization of myeloid *Mif* deficiency was performed through flow cytometric analysis on various myeloid cell populations. Selective breeding of the *Mif* $^{Fl/Fl}$ mice with the *LysMCre* mice for three generations yielded myeloid-specific *Mif* deficient mice. Genotypic PCR characterization of *Mif* $^{Fl/Fl}$ mice yielded expected band sizes of 404 bp while wild-type mice yielded an expected band size of 221 bp. Furthermore, genotypic characterization of *LysMCre* mice yielded an expected band size of 700 bp while wild-type mice yielded an expected band size of 350 bp. Phenotypic flow cytometric analysis of myeloid-specific *Mif* deficient mice revealed deletion of *Mif* in bone marrow-derived macrophages and peritoneal macrophages, but not in lymphoid cells. Our data indicate that selective breeding of *LysMCre* mice with our recently generated *Mif* $^{Fl/Fl}$ mice results in conditional *Mif* gene removal in myeloid cells in mice, which can help us gain a better understanding of the role *Mif* plays in the context of oral cancer and other inflammatory diseases.

1. Introduction

Macrophage migration inhibitory factor (*Mif*) encodes for a pro-inflammatory cytokine (*MIF)* involved in the innate immune system [1]. It has been observed that *MIF* downregulates p53, which in turn is linked to a pro-inflammatory and tumor-promoting response [2]. Furthermore, through the binding to the receptor CD74, *MIF* leads to the activation of the ERK1/ERK2 signaling pathway, which counteracts the effects of glucocorticoids by causing increased phospholipase 2 (PLA2), an up-regulator of inflammation [3,4]. Overall, *Mif* contributes to an increased inflammatory innate immune response.

Previously, it has been shown that global deletion of the *Mif* gene in mice (global *Mif* KO mice) leads to lower incidence and severity of oral cancer [5]. Moreover, another finding was the inhibition of myeloid cell-derived tumor-promoting cells in the global *Mif* KO mice [5]. Therefore, it is hypothesized that a mouse model in which *Mif* is conditionally deleted in only myeloid-derived cells will define the role of *Mif* in these cells during oral squamous cell carcinoma as well as other inflammatory diseases.

Nat L. Sternberg discovered the cre recombinase and loxP system through his hypothesis that the previously unexplained linearity of the P1 bacteriophage's recombination map was caused by site-specific recombination [6]. This event was seen to be caused by cre recombinase, a site-specific recombinase expressed by P1 [6,7]. It causes recombination by forming a covalent link with a DNA sequence called the loxP site that flanks the targeted gene (commonly referred to as floxing a gene) [8]. Through the covalent link, cre is then able to excise or invert the gene, depending on the orientation of the loxP sites [8]. Cre is now used in a plethora of ways, the

majority of which is to create targeted conditional knockouts of various genes [8]. This began when Brian Sauer, an accomplice of Sternberg, developed a mouse cell line expressing cre recombinase under the control of an inducible metallothionein promoter and, through the introduction of DNA fragments containing loxP sites, demonstrated that cre loxP mediated recombination can be achieved in an in vivo murine model [9]. In order to create these models, cre is inserted into the genome under the control of a promoter specific to the targeted tissue or cell type for deletion, and the resulting mouse is crossbred with a mouse with the floxed gene intended for removal [8]. The final mice will express both cre recombinase and the floxed gene as well as demonstrate the cre loxP mediated recombination of the targeted gene [8].

LysMCre mice, a knock-in mouse model that expresses cre recombinase under the control of the myeloid cell-specific *lysozyme 2* gene, developed by Clausen et al., is commonly used to generate myeloid-derived cell-specific conditional knockout mice [1010]. In previous literature, *LysMCre* mice have been used to develop a mouse model that demonstrates reduction of tumor development with myeloid cell-specific deletion of *inositol-requiring enzyme 1 alpha* in hepatocarcinoma [11]. Another of many examples of mouse models *LysMCre* mice were used in was to show how targeted macrophage downregulation of microsomal prostaglandin E2 synthase-1 can possibly be used as a way to treat and prevent cardiovascular inflammatory diseases [12]. All in all, the *LysMCre* mouse model allows for the creation of myeloid cell-specific targeted gene deleted mouse models that can be used in a wide variety of investigations.

Therefore, through the use of the *LysMCre* mouse with a mouse expressing the floxed *Mif* gene, myeloid-specific *Mif* deficient mice can be generated for future investigation of its effects on oral carcinoma.

2. Methods

2.1. Mouse Handling

C57BL/6 mice that were genetically modified to express the floxed *Mif* gene ($Mif^{Fl/Fl}$) was generated previously by Dr. Oghumu. C57BL/6 mice with a targeted mutation to express *Cre* recombinase within exon 1 of the lysozyme 2 gene [10] (*LysMCre*) were purchased from Jackson laboratory (JAX stock #004781). All mice were maintained in a 12 h day/night cycle, free access to food and water, and a temperature range held between 20-24 °C as well as a humidity range kept between 40-60%. These mice were monitored under the regulations upheld by the University Laboratory Animal Resources, which was approved by the Institutional Animal Care and Use Committee (Protocol #2018A00000054) as well as the Institutional Biosafety Committee of The Ohio State University.

2.2. Generation of Myeloid-Specific Mif Deficient Mice

The $Mif^{Fl/Fl}$ mice were bred with the *LysMCre* mice to generate the first filial generation (F1) $Mif^{Fl/+}$ $LysMCre^{+/-}$ heterozygous mice (Figure 1A). These heterozygous mice were bred together to derive F2 $Mif^{Fl/Fl}$ $LysMCre^{+/-}$ mice, which are the myeloid-specific *Mif* deficient mice (Figure 1B). From these F2 progeny mice, breeder pairs were selected with genotypes $Mif^{Fl/Fl}$ $LysMCre^{+/-}$ and $Mif^{Fl/Fl}$ $LysMCre^{-/-}$ mice. Offspring from this final breeder pair produced were myeloid-specific *Mif* deficient and wild-type mice at a 1:1 ratio.

(Figure 1)

2.3. Polymerase Chain Reaction and Agarose Gel Analysis

DNA was isolated from ear samples of the mice. Briefly, ear tissue was digested with Proteinase K, 20 mg/mL, (ThermoFisher Scientific) in SNET buffer. The DNA was isolated by an extraction through the use of 25:24:1 phenol: chloroform: isoamyl alcohol. Isopropanol then allowed for the precipitation of the DNA from its solubilized form, which was then isolated through centrifugation, washed with 70% ethanol, and reconstituted in TE buffer for future use.

When using the Polymerase Chain Reaction (PCR) to genotype the floxed *Mif* gene, the *Mif* $^{Fl/Fl}$ primer set was used for detecting the floxed gene (Table 1, Figure 2B) which provided an expected band size at 404bp for the mutant gene (Table 1, Figure 2B). For the wild-type *Mif* gene, the *Mif* WT primer set was used, which provided a band of 221bp (Table 1, Figure 2B). For PCR genotyping of *LysMCre*, the presence of *Cre* recombinase was detected using the *LysMCre* Mut primer set, which gave a band of 700bp as well as the *Cre* recombinase primer set, which provide a band of 100 bp (Table 1, Figure 2A). Lastly, the primer set used for detecting the wild-type *Lyz* gene used was *LysMCre* WT, which provided a 350bp band (Table 1, Figure 2A). All *Cre* primers were developed by The Jackson Laboratory [10] and *Mif* primers were developed previously by Dr. Oghumu. All Polymerase Chain Reactions were done using the OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (New England Biolabs). The *LysMCre* and *Mif* PCR cycle conditions used in genotyping are shown in Table 2 and 3, respectively. DNA samples were run with 6X TriTrack DNA Loading Dye (Thermo Scientific) and GeneRuler 50 bp DNA Ladder, ready to use (Thermo Scientific) was used for determining band sizes. Furthermore, for all *Mif* genotyping, 2% agarose gels were used, and, for *LysMCre* genotyping, 1.5% agarose gels were used. Ethidium bromide and a UV transilluminator were used to image the agarose gels.

Primer Set	Primer Name	Sequence (5'-3')	Expected Results (bp)
Mif Flox	*Mif* Common FW	GTTCCACCTTCGCTTGAGTC	404
	Mif flox RV	CTGCAAAGGGTCGCTACAG	
Mif WT	*Mif* Common FW	GTTCCACCTTCGCTTGAGTC	221
	Mif WT RV	AGAGCCTGGGCTTCCATT	
LysMCre Mut	*LysMCre* Common FW	CTTGGGCTGCCAGAATTTCTC	700
	LysMCre Mut RV	CCCAGAAATGCCAGATTACG	
LysMCre WT	*LysMCre* Common FW	CTTGGGCTGCCAGAATTTCTC	350
	LysMCre WT RV	TTACAGTCGGCCAGGCTGAC	

Table 1. Primers used for genotypic characterization

***LysMCre* Genotyping PCR Conditions**			
Step	**Temp °C**	**Time (min:sec)**	**Notes**
1	94	2:00	
2	94	0:20	
3	65	0:15	-0.5 °C decrease per cycle
4	68	0:20	
5	--	--	Repeat steps 2-4 for 10 cycles (Touchdown Protocol)

6	94	0:15	
7	60	0:15	
8	68	0:45	
9	--	--	Repeat steps 6-8 for 28 cycles
10	68	5:00	
11	10	--	

Table 2. PCR conditions used for genotypic characterization of *LysMCre*

***Mif* Genotyping PCR Conditions**			
Step	**Temp °C**	**Time (min:sec)**	**Notes**
1	94	3:00	
2	94	0:40	
3	47	0:40	
4	68	0:40	
5	--	--	Repeat steps 2-4 for 29 cycles
6	68	5:00	
7	10	--	

Table 3. PCR conditions used for genotypic characterization of *Mif*

2.4. Flow Cytometric Analysis

Postmortem, we harvested the following organs or cell samples from the mice: spleens, lymph nodes, thioglycollate-elicited peritoneal macrophages and bone marrow. Collected spleens and lymph nodes were passed through a 70 μm nylon mesh, generating a single cell suspension of these organ samples. Splenocytes were treated for 3 min with ACK Lysis Buffer to lyse erythrocytes before further use. To stimulate thioglycollate-elicited peritoneal macrophages, mice targeted for sacrifice were given 1 mL intraperitoneal injections of 3.8% brewer's thioglycollate made from Thioglycollate Broth (Sigma-Aldrich, St. Louis, MO, USA) 3 days prior to terminal sacrifice. Once the mice were euthanized, 5 mL of cold PBS was injected peritoneally to wash the cavity and then collected in order to extract the thioglycollate-elicited peritoneal macrophages. Lastly, bone marrow was extracted from the femurs and tibias of the mice and the erythrocytes within those samples were also lysed with ACK Lysis Buffer for 3 minutes. They were then seeded on 24-well plates with media supplemented with 15% granulocyte macrophage colony stimulating factor (GM-CSF) or colony stimulating factor 1 (CSF-1), which gave rise to bone marrow-derived dendritic cells and macrophages, respectively. 3 days post, the cells were supplemented with an additional 75 μL of 15% GM-CSF or CSF-1 treated media. 5-7 days post initial plating, these cells were scraped for analysis. 10^6 cells from each organ sample were incubated with 1 μg/mL CD11b or CD4 PE anti-mouse conjugated monoclonal antibodies (BD Pharmingen). Furthermore, the cells were permeabilized using intracellular staining permeabilization wash buffer and stained intracellularly with 0.2 μg/mL Rabbit anti-MIF primary antibodies (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA) diluted followed by AF647 conjugated goat anti-rabbit IgG secondary antibodies at a

concentration of 1 µg/mL. The flow cytometric data was analyzed through FlowJo (Tree Star Inc., Ashland, OR, USA).

3. Results

3.1 Breeding Scheme

In order to generate myeloid-specific *Mif* deficient mice, mice homozygous for the loxP flanked *Mif* gene (*Mif* $^{FL/FL}$) were bred with mice homozygous for Cre recombinase within the lysozyme 2 gene or *LysMCre* $^{+/+}$ (Figure 1A). The resulting pups that were all heterozygous for both floxed *Mif* and *LysMCre* were then crossbred (Figure 1B). The resulting progeny determined to be homozygous for floxed *Mif* and heterozygous for *LysMCre* were 7.89 % (3/38) of the total mice produced. These were the myeloid-specific *Mif* deficient mice. Mice homozygous for floxed *Mif* and wild-type for the *Lyz* gene were 13.16% (5/38) of the total mice produced. These were used as wild-type controls (Figure 1C).

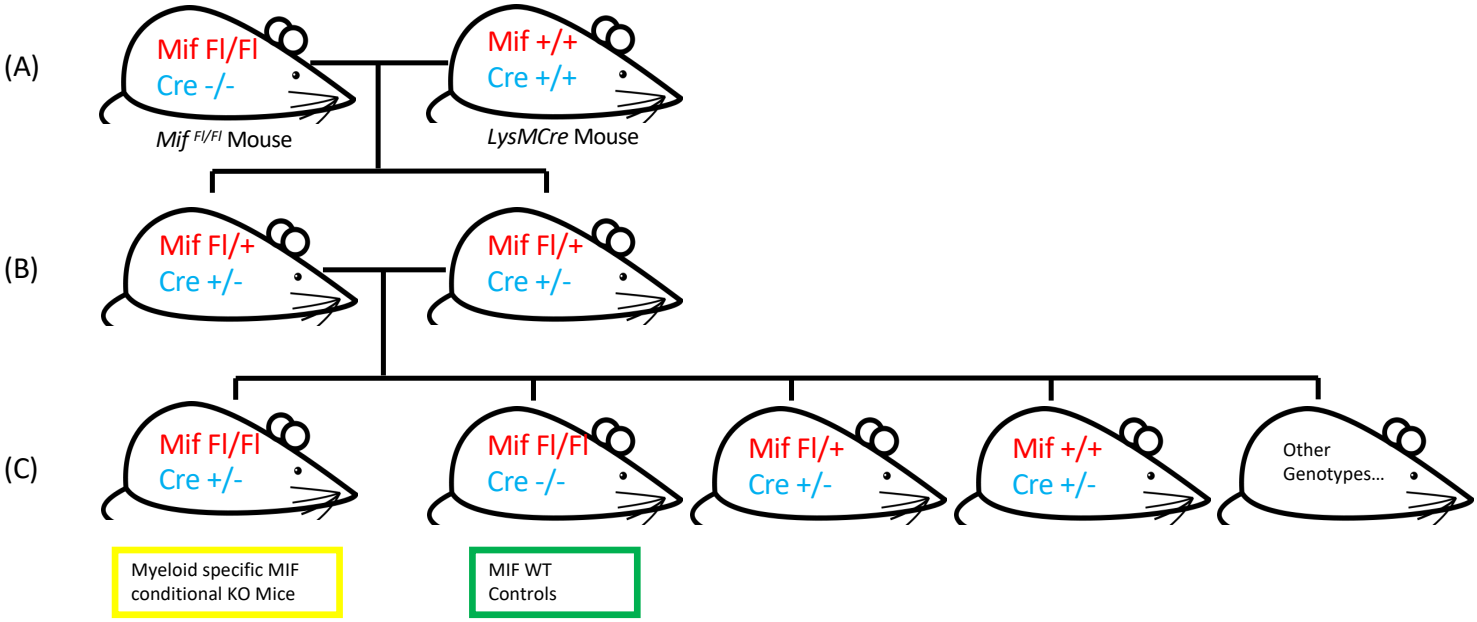

Figure 1. Breeding strategy used to generate Myeloid-specific *Mif* KO mice. **(A)** P1, the parental generation. **(B)** F1, the first filial generation. **(C)** F2, the second filial generation.

Generation	Genotype	Frequency	Percentage	Expected Percentage
F1	Mif Fl/+ Cre +/-	15/15	100	100
F2	Mif Fl/Fl Cre +/-	3/38	7.89	12.5
	Mif Fl/Fl Cre -/-	5/38	13.16	6.3
	Mif Fl/+ Cre +/-	12/38	31.58	25
	Mif +/+ Cre +/-	4/38	10.53	12.5
	Mif Fl/+ Cre +/+	2/38	5.26	12.5

	Mif Fl/+ Cre -/-	4/38	10.53	12.5
	Mif Fl/Fl Cre +/+	0/38	0	6.3
	Mif +/+ Cre +/+	6/38	15.79	6.3
	Mif +/+ Cre -/-	2/38	5.26	6.3

Table 4. Frequencies, percentages, and expected percentages of all of the genotypes from the F1 and F2 generations.

3.2 Optimization of Mif $^{Fl/Fl}$ Genotyping Protocol

In order to ensure clear results in the genotyping of *Mif* $^{Fl/Fl}$, the PCR protocol was optimized through the use of gradient PCR. In gradient PCR, the optimal temperature for any step can be found by running the same sample at various temperatures. The most critical step is the annealing of the primers, which can be optimized in order to reduce nonspecific binding. By running a range of annealing temperatures from 38 to 63°C on both the wild-type and mutant assays of *Mif* genotyping protocols, the ideal annealing temperature for both primer pairs was determined to be 47.4°C (Figure 5).

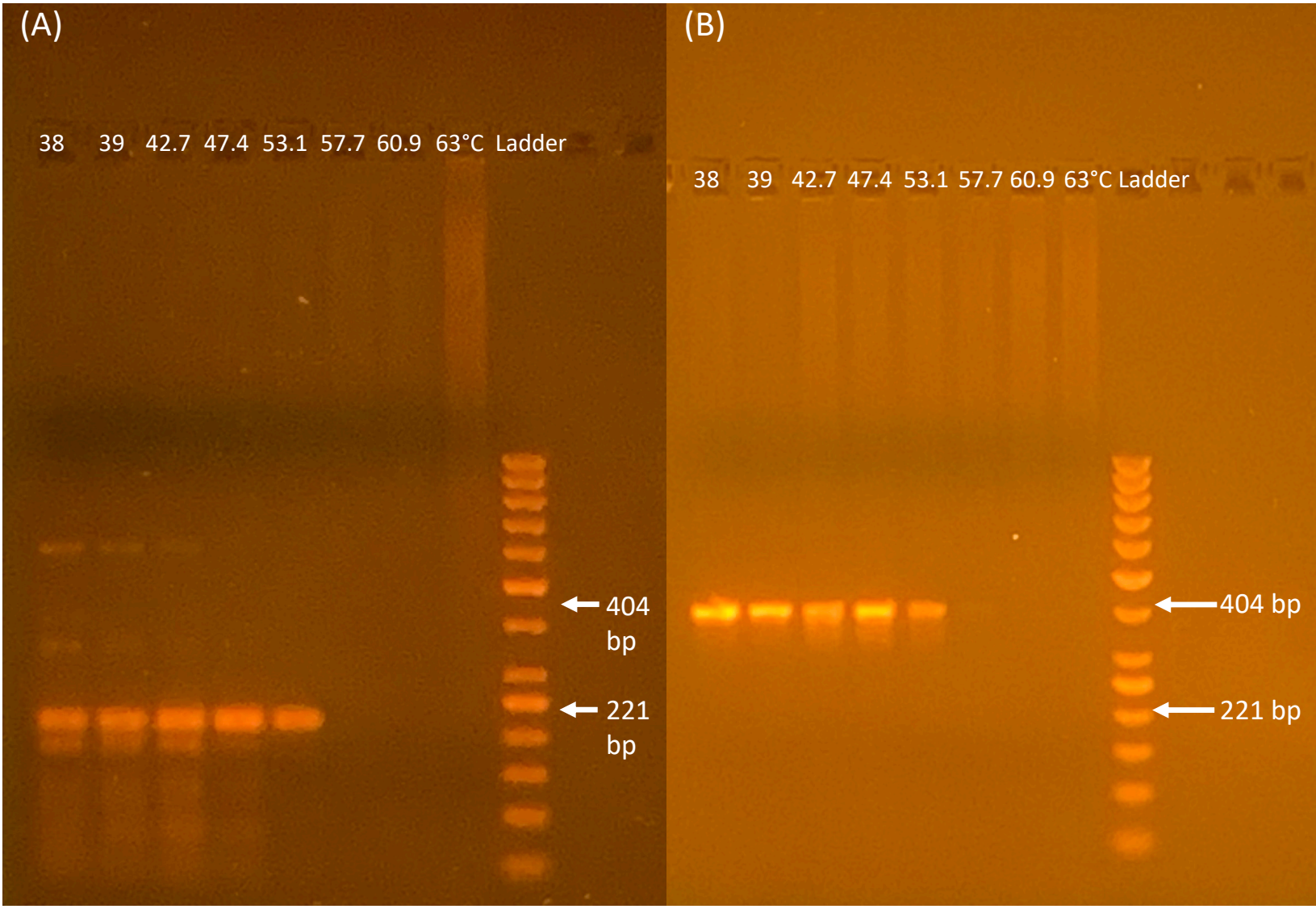

Figure 2. (A) Gradient PCR optimization of *Mif* WT assay using a *Mif* +/+ DNA sample with the annealing temperature range of 38-63 °C. **(B)** Gradient PCR optimization of *Mif* Mutant assay using a *Mif* fl/fl DNA sample with the annealing temperature range of 38-63 °C.

3.3 Genotypic Characterization of LysMCre

Throughout the selective breeding process, genotypic characterization by PCR and agarose gel electrophoresis was used to determine the mice selected for subsequent breeding, as described in the breeding scheme. For detecting the presence of Cre in the *Lyz* gene, two primer sets were used. The *LysMCre* Mut primer set, comprised of *LysMCre* Common FW and *LysMCre* Mut RV, provided a band of 700 bp to demonstrate the presence of Cre (Figure 2A). Secondly, the *LysMCre* WT primer set, containing *LysMCre* Common FW and *LysMCre* WT RV, showed a DNA band size of 350 bp, which was used to indicate the wild-type *Lyz* gene (Figure 2A). Throughout the selective breeding process, three different *LysMCre* genotypes were possible. First, *LysMCre* $^{+/+}$ mice, which express *LysMCre* in both alleles of the *Lyz* gene,

showed bands with only the *LysMCre* Mut primer set (Figure 3). *LysMCre* $^{-/-}$ mice or mice wild-type for the *Lyz* gene display bands with only the *LysMCre* WT primer set (Figure 3). Lastly, *LysMCre* $^{+/-}$ mice who only express *LysMCre* in one allele of the *Lyz* gene exhibit bands with both *LysMCre* Mut and *LysMCre* WT primer sets (Figure 3).

3.4 Genotypic Characterization of Mif $^{Fl/Fl}$

Similar to the genotyping of *LysMCre,* the detection of the loxP sequences flanking the *Mif* gene was done with two primer sets. Comprised of *Mif* Common FW and *Mif* Flox RV, the *Mif* Flox primer set provided a band size of 404 bp in the presence of the floxed *Mif* gene (Figure 2B). On the other hand, the *Mif* WT primer set, which contains *Mif* Common FW and *Mif* WT RV, gave a band size of 221 bp in the presence of the wild-type *Mif* gene (Figure 2B). Analogous to the *LysMCre* genotyping, three different genotypes of the *Mif* gene were obtainable throughout the breeding experiment. Mice homozygous for floxed *Mif*, or *Mif* $^{Fl/Fl}$, displayed bands only through the use of the *Mif* Flox primer set (Figure 4). *Mif* $^{Fl/+}$ mice that expressed the loxP sites in only one allele of the *Mif* gene showed bands with both the *Mif* WT and *Mif* Flox primer sets (Figure 4). Finally, *Mif* $^{+/+}$ mice wild-type for the *Mif* gene expressed DNA bands only with the *Mif* WT primer set (Figure 4).

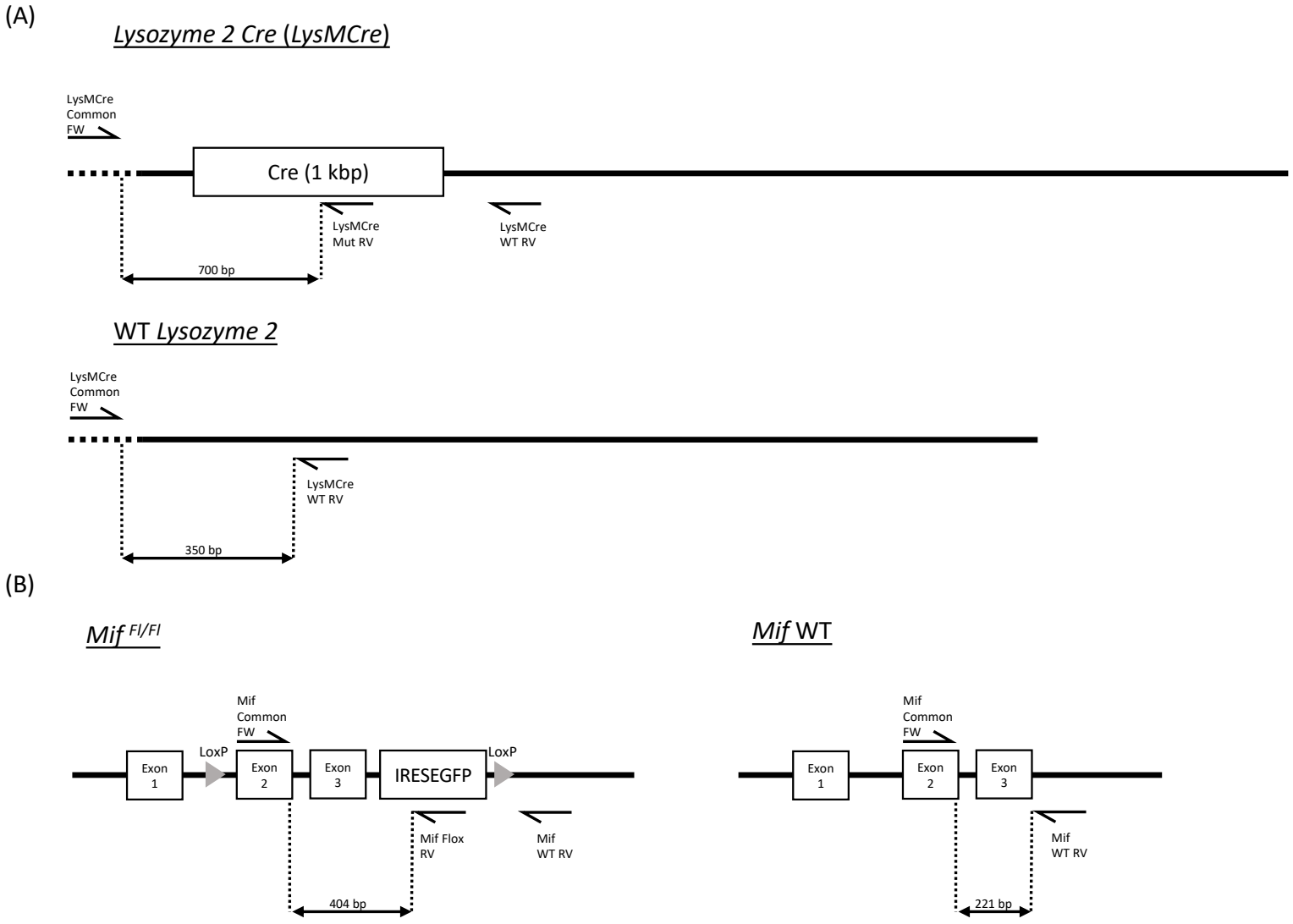

Figure 3. (A) Gene map for *LysMCre* and *Lyz* WT locus and location of primers used for genotyping mice. **(B)** *Mif* $^{Fl/Fl}$ and *Mif* WT gene maps and location of primers for genotyping mice.

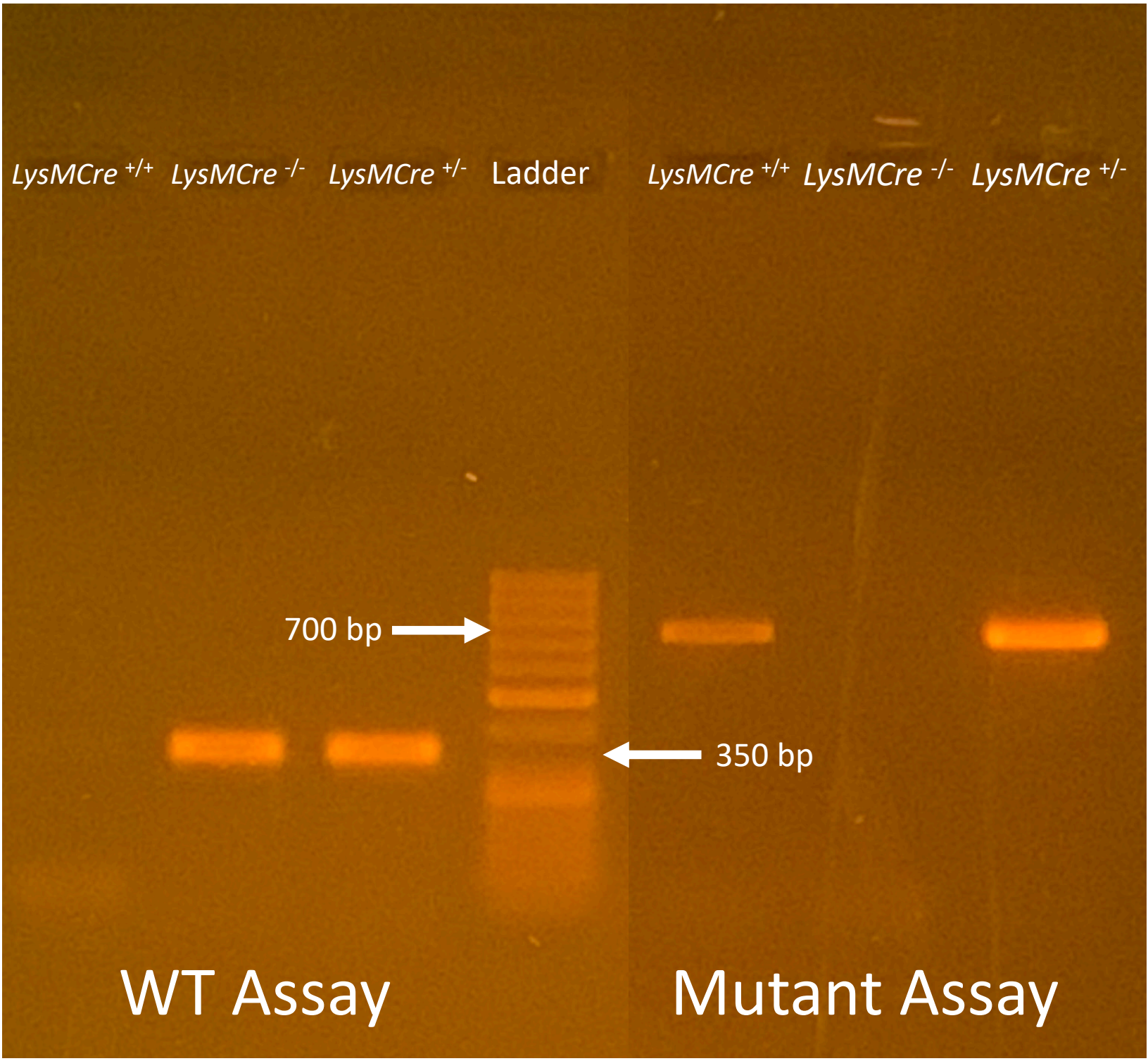

Figure 4. *LysMCre* genotyping examples with *LysMCre* mouse, WT mouse, and heterozygous mouse DNA samples in both the WT and mutant assays.

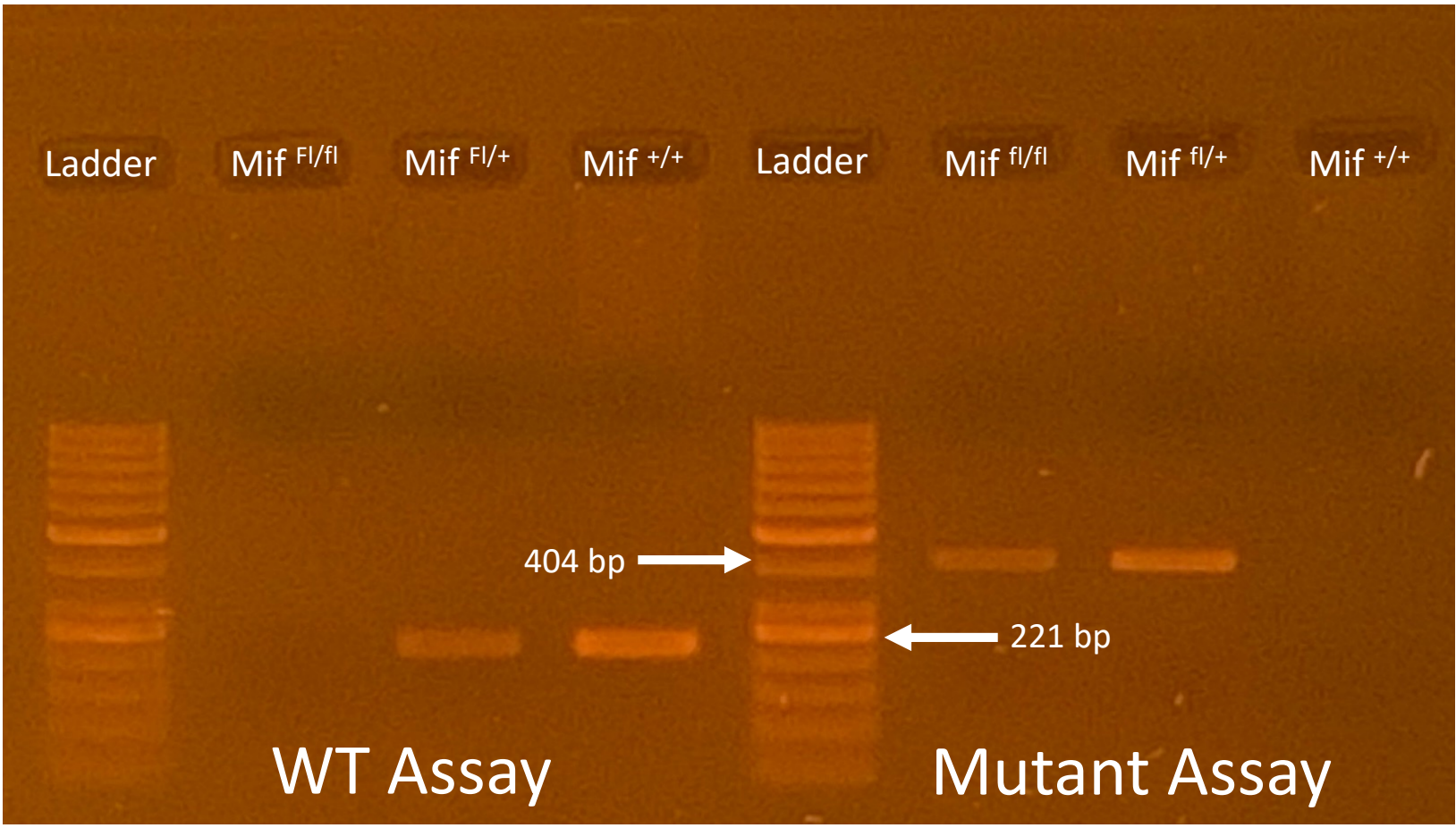

Figure 5. *Mif* genotyping examples with *Mif* fl/fl, *Mif* fl/+, and *Mif* +/+ mouse DNA samples in both the WT and mutant Assays.

3.5 Phenotypic Characterization

Flow cytometric analysis was used to visualize phenotypic differences in the expression of *Mif* in bone marrow-derived cells. The three types of mice that were investigated phenotypically were the myeloid-specific *Mif* deficient mice, *Mif* wild-type mice, and global *Mif* knockout mice. The CD11b expressing bone marrow-derived macrophages (BMDMS) as well as the CD11b expressing bone marrow-derived dendritic cells (BMDCs) both saw a decrease in *Mif* expression in the myeloid-specific *Mif* deficient mice as compared to the *Mif* wild-type mice (Figure 6). The CD11b expressing BMDCs however had a smaller decrease in *Mif* as compared to the BMDMs, which had similarly decreased levels of expression as the global *Mif* knockout (Figure 6).

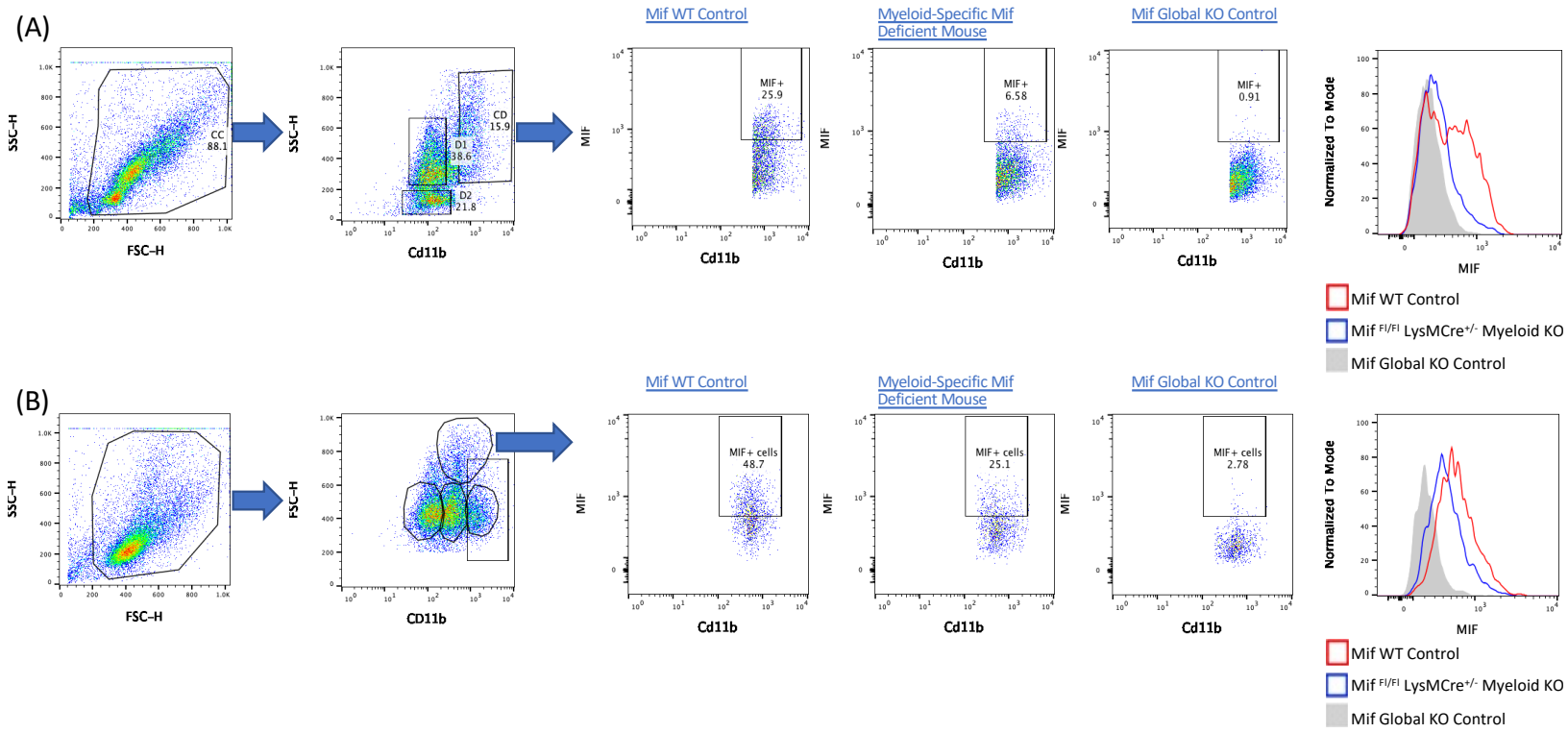

Figure 6. (A) Flow cytometric analysis of bone marrow-derived macrophages. **(B)** Flow cytometric analysis of bone marrow-derived dendritic cells

Thioglycollate-elicited peritoneal macrophages (PMs), lymph node-derived cells (LNs), and splenocytes (SPs) were all other various organs also phenotypically analyzed for differences in *Mif* expression. Similar to the BMDCs, the CD11b expressing PMs from the myeloid-specific *Mif* deficient mice had a slight decrease in *Mif* expression, but not to the level of the global *Mif* knockout (Figure 7A). In order to demonstrate the decreased *Mif* expression is myeloid cell-specific, the *Mif* expression in the CD4 expressing populations in the LNs and SPs were compared across the same sample group. We determined, no notable difference in those populations between the myeloid-specific *Mif* deficient mice and the *Mif* wild-type mice (Figure 7B and 7C). Furthermore, the global *Mif* knockout maintained decreased levels of expression across all populations (Figures 6 and 7).

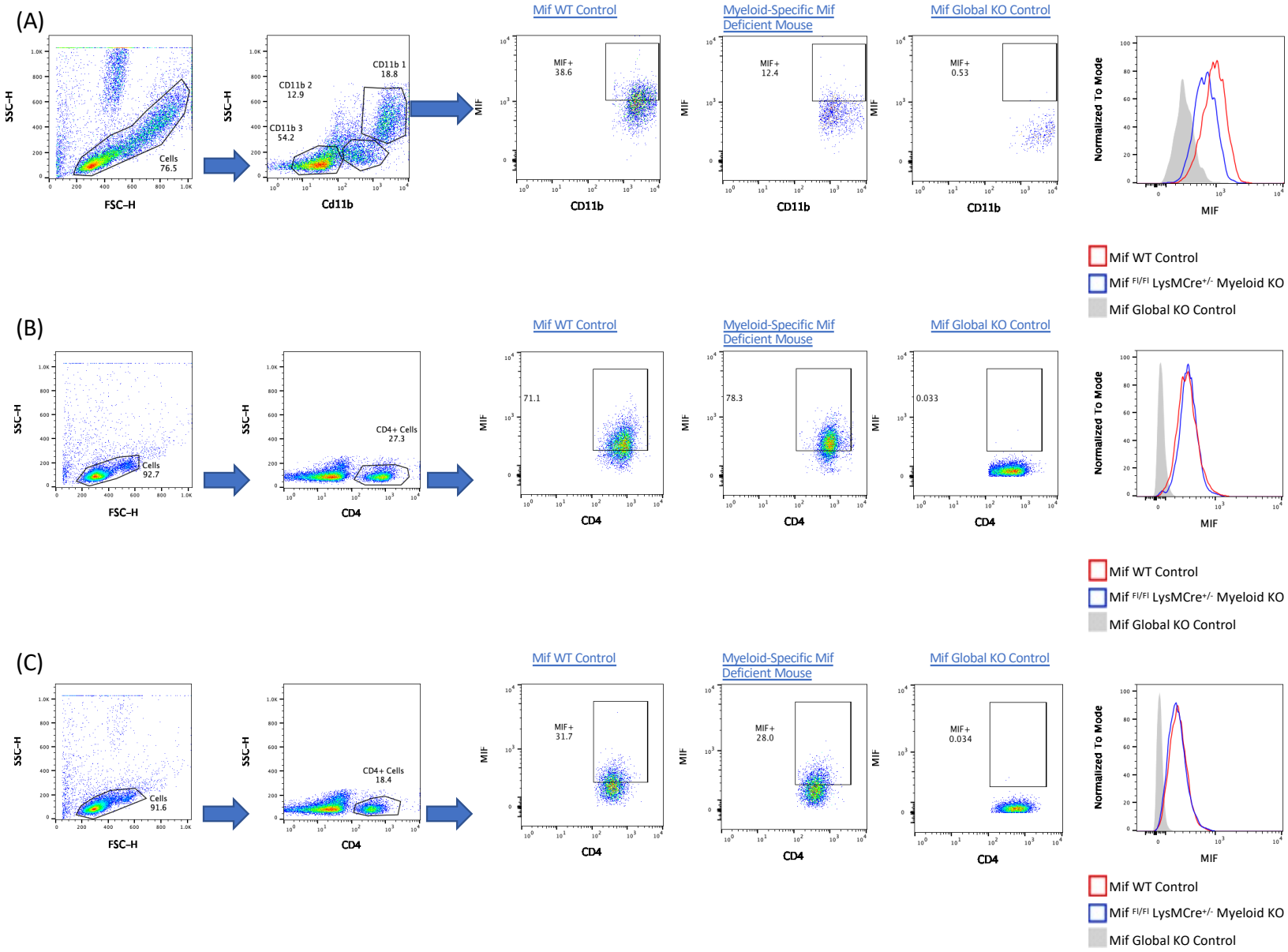

Figure 7. (A) Flow cytometric analysis of thioglycollate-elicited peritoneal macrophages. **(B)** Flow cytometric analysis of lymph node-derived Cells. **(C)** Flow cytometric analysis of splenocytes.

4. **Discussion**

Here, we demonstrate the creation of myeloid-specific *Mif* deficient mice. This was achieved through the crossbreeding of a knock-in mouse expressing cre recombinase under the control of the *Lysozyme 2* promoter and a mouse expressing the floxed *Mif* gene. Genotypic and phenotypic analysis with PCR and agarose gel electrophoresis as well as flow cytometry, respectively, were used to confirm the findings. With the creation of these mice, further studies can be undertaken to explore the effects of *Mif* deletion on oral carcinoma as well as in other inflammatory diseases.

Previously, *LysMCre* mice have been used in a plethora of studies to examine the effects of the deletion of various genes in myeloid cells. An investigation on the effects of the *Slc2a1* myeloid-specific deletion as well as a study on the effects of *ARTD1* myeloid-specific deletion both showed the use of BMDMs and PMs as a way to demonstrate the deletion of the genes as well as their effects [13,14]. Various other publications used other methods of confirming the cre mediated deletion of their genes as well. Western blotting and cell specific genotyping was another tactic to demonstrate gene deletion employed by Takeda et al. when investigating *STAT3* deletion in myeloid cells and its effect on enterocolitis [15]. It has been seen that the *LysMCre* mice have been used in a wide range of studies demonstrating its capabilities to create a myeloid specific targeted gene knockout mouse model.

In the breeding scheme of the mice, although the myeloid-specific *Mif* knockout mouse was achieved, an improved scheme can be proposed. Other studies have used the strategy of backcrossing the heterozygous flox and heterozygous cre mice in the first filial generation with the homozygous flox parent mouse to achieve a higher chance of getting the desired mouse [16]. Although this change is not necessary for the creation of these mice, it can be made in future studies to expedite the creation of future mouse models.

In the genotypic characterization of the mice, the optimization of the $Mif^{Fl/Fl}$ genotyping protocol allowed for clear results. It can be observed in figures 3 and 4 with each possible genotype shown that with these improvements, the genotypes of the mice were easily obtained and confirmed.

In the phenotypic characterization, the reduction in *Mif* expression was tested through various organs and by isolating CD11b and CD4 expressing population as an example of myeloid cell populations and non-myeloid cell populations, respectively. The decrease in *Mif* expression can be seen in the CD11b expressing populations from the BMDMs, BMDCs, and PMs. This confirmed the excision of *Mif* through cre recombinase. Furthermore, this change in *Mif* expression was not seen in the CD4 expressing populations of the splenocytes and lymph node-derived cells. An interesting finding from this analysis was the lack of complete deletion of *Mif* in certain myeloid cell populations, as depicted in figures 6B and 7A. Although there was a reduction in *Mif,* there were still populations of CD11b cells that were expressing *Mif* in the BMDC and PM populations. This lack of entire deletion of *Mif* is analogous with other studies using the *LysMCre* model as well. Takeda et al. observed a residual expression of *STAT3* following *LysMCre* mediated deletion in the cell-specific PCR and western blotting of their PMs [15]. As observed through southern blotting by the team that developed the *LysMCre* mice, *LysMCre* is able to achieve 79% deletion in BMDMs and 31% in BMDCs, which explains the lack of entire deletion in our data as well [10]. Furthermore, our observations could also mean that there are certain CD11b cells that do not express the *Lysozyme 2* gene and still express *Mif*, which could be investigated in future studies. Overall, the phenotypic characterization of the mice demonstrated and confirmed the myeloid cell-specific deletion of *Mif*.

Through the use of genotypic characterization, mice resulting from the crossbreeding of the *LysMCre* and $Mif^{Fl/Fl}$ mice were able to be identified and the myeloid-specific *Mif* deficient mice were able to be selected out. With the optimization of the $Mif^{Fl/Fl}$ genotyping protocol, the identification of these mice was improved and made clearer for the team. Using flow cytometric analysis of CD11b populations in BMDMs, BMDCs, and PMs as well as CD4 populations in LNs and SPs; the myeloid-specific *Mif* deficient mice were phenotypically characterized to demonstrate cre mediated decreased levels of *Mif* in only myeloid cells. Through the creation of

this mouse model, the role of *Mif* deletion during oral cancer and other inflammatory diseases can be investigated.

References

1. Calandra, T., & Roger, T. 2003. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol 3*(10): 791–800. https://doi.org/10.1038/nri1200
2. Hudson, J. D., Shoaibi, M. A., Maestro, R., Carnero, A., Hannon, G. J., & Beach, D. H. 1999. A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J Exp Med* 190(10): 1375–82. https://doi.org/10.1084/jem.190.10.1375
3. Mitchell, R. A., Metz, C. N., Peng, T., & Bucala, R. 1999. Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. *J Biol Chem* 274(25): 18100–06. https://doi.org/10.1074/jbc.274.25.18100
4. Leng, L., Metz, C. N., Fang, Y., Xu, J., Donnelly, S., Baugh, J., Delohery, T., Chen, Y., Mitchell, R. A., & Bucala, R. 2003. MIF signal transduction initiated by binding to CD74. *J Exp Med* 197(11): 1467–76. https://doi.org/10.1084/jem.20030286
5. Oghumu, S.; Knobloch, T.J.; Terrazas, C.; Varikuti, S., Ahn-Jarvis, J., Bollinger, C.E., Iwenofu, H., Weghorst, C.M. and Satoskar, A.R. 2016. Deletion of macrophage migration inhibitory factor inhibits murine oral carcinogenesis: Potential role for chronic pro-inflammatory immune mediators. *Int J Cancer* 139: 1379-90. https://doi.org/10.1002/ijc.30177
6. Yarmolinsky, M.; Hoess, R. 2015. The legacy of nat sternberg: the genesis of cre-lox technology. *Annu Rev Virol* 2(1): 25–40. https://doi.org/10.1146/annurev-virology-100114-054930
7. Nagy, A. 2000. Cre recombinase: The universal reagent for genome tailoring. *Genesis* 26: 99-109. https://doi.org/10.1002/(SICI)1526-968X(200002)26:2<99::AID-GENE1>3.0.CO;2-B
8. Kos CH. 2004. Methods in Nutrition Science: Cre/loxP System for Generating Tissue-specific Knockout Mouse Models. *Nutr Rev* 62(6): 243-46. https://doi.org/10.1111/j.1753-4887.2004.tb00046.x
9. Sauer, B.; Henderson, N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc Natl Acad Sci USA* 85(14): 5166-70. https://doi.org/10.1073/pnas.85.14.5166
10. Clausen BE; Burkhardt C; Reith W; Renkawitz R; Forster I. 1999. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res* 8(4): 265-77 PubMed: 10621974MGI: J:67924
11. Van Campenhout, S.; Tilleman, L.; Lefere, S.; Vandierendonck, A.; Raevens, S.; Verhelst, X.; Geerts, A.; Van Nieuwerburgh, F.; Van Vlierberghe, H.; Devisscher, L. 2020. Myeloid-specific IRE1alpha deletion reduces tumour development in a diabetic, non-alcoholic steatohepatitis-induced hepatocellular carcinoma mouse model. *Metab.: Clin Exp* 107: 154220. https://doi.org/10.1016/j.metabol.2020.154220
12. Chen, L.; Yang, G.; Jiang, T.; Tang, S. Y.; Wang, T.; Wan, Q.; Wang, M.; FitzGerald, G. A. 2019. Myeloid Cell mPges-1 Deletion Attenuates Mortality Without Affecting Remodeling After Acute Myocardial Infarction in Mice. *J Pharmacol Exp Ther* 370(1): 18–24. https://doi.org/10.1124/jpet.118.256057
13. Freemerman AJ; Zhao L; Pingili AK; Teng B; Cozzo AJ; Fuller AM; Johnson AR; Milner JJ; Lim MF; Galanko JA; Beck MA; Bear JE; Rotty JD; Bezavada L; Smallwood HS; Puchowicz MA; Liu J; Locasale JW; Lee DP; Bennett BJ; Abel ED; Rathmell JC; Makowski L. 2019. Myeloid Slc2a1-Deficient Murine Model Revealed Macrophage Activation and Metabolic Phenotype Are Fueled by GLUT1. *J Immunol* 202(4): 1265-1286. PubMed: 30659108MGI: J:272884
14. Kunze FA; Bauer M; Komuczki J; Lanzinger M; Gunasekera K; Hopp AK; Lehmann M; Becher B; Muller A; Hottiger MO. 2019. ARTD1 in Myeloid Cells Controls the IL-12/18-IFN-gamma Axis in a Model of Sterile Sepsis, Chronic Bacterial Infection, and Cancer. *J Immunol* 202(5): 1406-1416 PubMed: 30674576 MGI: J:273001
15. Takeda K; Clausen BE; Kaisho T; Tsujimura T; Terada N; Forster I; Akira S. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 10(1):39-49 PubMed: 10023769 MGI: J:53681
16. Li K, Zhang Y, Liang KY, Xu S, Zhou XJ, Tan K, Lin J, Bai XC, Yang CL. 2017. Rheb1 deletion in myeloid cells aggravates OVA-induced allergic inflammation in mice. *Sci Rep* 7:42655. PubMed: 28225024MGI: J:271919